ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5300

The occurrence of Myxosoma cerebralis spores in individual organs of the California trout

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Original title: Nalaz spora Myxosoma cerebralis u pojedinim organima Kalifornijskih pastrva

Ref: Veterinaria (Sarajevo) 30(1): 59-64, 1981

Original language: Serbo-Croatian

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1987

12 typescript pages

Secretary of State Secrétariat d'État

90-02115/

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

Translated from - Traduction de	Into - En
Serbo-Croatian	English

Author - Auteur

Sead Gradjanin and Dr Midhat Cankoviċ

Title in English or French - Titre anglais ou français

THE OCCURRENCE OF MYXOSOMA CEREBRALIS SPORES IN INDIVIDUAL ORGANS OF THE CALIFORNIA TROUT

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

NALAZ SPORA MYXOSOMA CEREBRALIS U POJEDINIM ORGANIMA KALIFORNIJSKIH PASTRVA

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Veterinaria

Reference in English or French - Référence en anglais ou français

Veterinary medicine

Publisher - Éditeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
	Year / Année	Volume	Issue No. / Numéro	59–64
Place of Publication / Lieu de publication				Number of typed pages / Nombre de pages dactylographiées
[Yugoslavia]	1981	30	1	12

Requesting Department / Ministère-Client: DFO

Branch or Division / Direction ou Division: IPB

Person requesting / Demandé par: M. Palmer

Your Number / Votre dossier nº:

Date of Request / Date de la demande: November 25, 1986

Translation Bureau No. / Notre dossier nº: 2894118

Translator (Initials) / Traducteur (Initiales): MW

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Canada

SEC 5-111 (84-10)

Secretary of State / Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU / BUREAU DES TRADUCTIONS

Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
	DFO	IPB	Ottawa, Ontario
Bureau No.–N° du bureau	Language – Langue	Translator (Initials) – Traducteur (Initiales)	
2894118	Serbo-Croatian	MW	JAN 20 1987

Source: Veterinaria (Veterinary medicine), Vol. 30, No. 1, 1981, pp. 59-64 [Yogoslavia]

UDC 619:593.194:637.56

THE OCCURRENCE OF MYXOSOMA CEREBRALIS SPORES IN INDIVIDUAL ORGANS OF THE CALIFORNIA TROUT

by

Sead Gradjanin and Dr Midhat Cankovič*

Introduction

Due to the constantly increasing demand for animal protein and /59** in order to satisfy the general requirements of modern nutrition, comestible fish species are frequently removed from the ecosystem in which they occur naturally and introduced into new areas. Such has been the case in Yugoslavia with the California trout (Salmo gairdneri irideus Gibb.), which is being raised intensively at salmonid fish farms

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**Numbers in the right hand margin refer to the corresponding page in the Serbo-Croatian text.

as a commercial, comestible fish. This and other such transfers of fish from one environment to another can also transfer the fish's parasites as well and in this manner enable them to spread in a new environment. Or else the fish can be invaded by parasites indigenous to the new environment. In either case the consequences can be very serious. Given a case of intensive production, in which the situation requires a high population density and frequent feeding chiefly with artificial food combined with a reduction in the resistance of the fish, parasitic diseases display a tendency to spread rapidly and to produce considerable losses. Salmonid young at fish farms and in open water are particularly threatened by parasites of the phylum Protoza. Parasites in this category include Myxosoma (Lentospora) cerebralis, family Myxosomatidae Poche, 1913, class Cnidosporidia Doflein, 1901.

Even though this disease was identified in Germany as early as 1903, there is very little information in Yugoslav or foreign literature on the parasite's biological cycle, epizootiology, pathological changes in anatomy, or on the possibilities for suppressing this insidious disease. The available research results indicate that the disease has usually been suppressed by direct methods--by veterinary intervention, and this has been true in Yugoslavia as well as abroad.

Whirling sickness [myxosomatosis] was first described in Yugoslavia by Kiškarolj et al. [1970] at the Jezero Fish Farm near Jajce. The first symptoms of the disease were noted in young fish that were four months old. A histological examination of individual immatures that exhibited symptoms of this disease indicated that the spores were five months old. Snoj [1973] states that whirling sickness cannot be spread through the eggs. Spores were found in the spleen and in the kidneys of the fish that were examined. It was found that the

multiplication of the causative agent begins in the stomach, while well-developed forms were also found among the contents of the intestine. The spores were found in the ooze of basins after infested juvenile fish had been maintained in them for a few weeks. Uspenskaya [1957] has described myxosomatosis at a fish farm. The first symptoms of the disease were noted at the end of June and at the beginning of July, while the first spores were found in mid-August. Since spores were found later in different tissues, the author is of the opinion that infected fish can secrete spores while they are still alive and that the spores are not liberated simply by the decay of cartilage. The quantity of spores constantly increases in September and October, while the infested juvenile is only one-fifth to one-sixth the weight of a healthy juvenile from the same fish pond. Bauer [1959] draws a distinction between different forms of the disease. There is acute development, in which the majority of the juveniles can perish [rapidly] without any external symptoms. A second form produces uncoordinated movements and circling. The third form is a less dangerous form in which the tail blackens and in which various skeletal deformations subsequently appear. As time passes, the trout recover from the disease--even the blackness of the tail can disappear, but they make less beneficial use of their food and they develop slowly and poorly. Christensen [1966] states that the spores retain their capacity to invade even after more than fifteen years. Šuljman [1966] has identified two stages of development in the biological cycle of M. cerebralis. The plasmodial stage is chiefly found towards the end of the fish's first year, while later, when bone formation is under way, only spores are found. The size of the spores ranges from 6 to 10 microns. Hoffman [1961] provides a description of the spread of myxosomatosis, the manner of identifying the disease, the

etiology, the source and reservoir of infestation, the manner of contagion, the incubational period, sensitivity and resistance, and preventive measures for suppressing this disease. According to this same author, under experimental conditions the symptoms of this disease can appear as soon as the end of two weeks, while at fish farms the first signs of the disease are observed after one or two months of infestation.

Myxosoma cerebralis produces round or oval spores, which were first identified by Hofer in 1903. The diameter is approximately 7.5 microns and within the spore there are two polar capsules and an ameboid embryo. Young trout are invaded perorally. Each polar capsule liberates a single polar filament in the intestine of the trout, which the spore uses to attach itself firmly to the wall of the intestine. A vegetative form emerges from the ameboid embryo--a trophozoite that manages to enter the cartilage through the blood or the lymphs. As a result of their growth and their direct division, the trophozoites ravage the cartilage, which most often results in deformities such as: curvature of the spine; shortening of the tail section of the body; and the formation of a so-called "frog head" as a result of deformed head bones. A dark pigmentation in the tail section of the body is readily observed in a significant number of diseased fish because of the pressure of the deformed vertebra of the spinal column on the sympathetic nerve. Diseased and dead specimens are the chief source of contagion.

Myxosomatosis, which is better known as "trout whirling sickness," not only results in a large number of deaths among young trout, it also results in poorer growth, and in increased sensitivity to other parasites and to reproductive and contagious diseases.

Bearing in mind the harm and trouble that myxosomatosis causes for Yugoslav salmonid fish farming, and in order to become better acquainted with the course of infestation and its epizootiology, the goal of researchers at the Institute for Parasitology and Invadive Disease was to study not only the bone-cartilage skeleton of the head, but also the extent and intensity of infestation by spores of Myxosoma /61
cerebralis of the other organs--the brain, the liver, the spleen, and the kidneys--and the contents of the intestine, and all this at various times in the first year of the fish's life after the first clinical manifestation of the disease.

Material and Method

Four samples, each of 30 specimens of juvenile California trout that exhibited clinical signs of myxosomatosis were examined by the native preparation method in the period between July and December. The bone-cartilage skeleton of the head, the brain, the liver, the spleen, the kidneys, and the contents of the intestine were utilized for the spore examination.

We carried out dissection and the microscopic examination in the following manner: we cut off the head with scissors slightly behind the branchial openings. We then cut off the lower jaw and removed the eyes with tweezers before making a longitudinal incision alsong the middle of the head between the eye sockets. After the skin was removed with tweezers and the brain set aside, we placed the cartgilage-bone remnant of the head on a slide together with one or two drops of water. After we had sliced this up very finely with a scalpel, we removed any large particles from the macerate with curved tweezers and covered what remained with a cover glass. We conducted the examination using a

Reichert microscope with a 45-power objective and a 10-power eyepiece. We saw the spores among the particles like pin heads, which glittered characteristically when the microscrew of the microscope was turned. The finding was verified with a 60-power objective with which we could see the structure and shape of the spore with the polar capsule and the ameboid embryo. We examined the brain, the liver, the spleen, the kidneys, and the contents of the intestine of the fish in the same manner.

We estimated the intesity of infestation as follows: we characterized a finding of 1-5 spores in the field of vision as mild; a finding of 5 to 10 as moderate; and a finding of 11 or more spores as a severe infestation.

Results

After the first discovery of clinical manifestations of whirling sickness, we carried out a test of the cartilage-bone tissue from the head, the brain, the liver, the spleen, and the kidneys, and of the contents of the intestine by the native preparation method in order to find spores of Myxosoma cerebralis. In the period between July and December (July, August, October, and December), we examined four randomly chosen samples, each consisting of 30 clinically diseased trout.

During the study made in the month of July, we found spores in the head skeletons of 12 specimens, which included eight cases of moderate infestation and four of mild intensity. Spores were also found in the brain of nine of the 12 positive trout specimens. The infestation was found to be moderate in two cases and it was weak in seven cases. Furthermore, spores were also found in the kidneys in two

trout and in the kidneys and the bone-cartilage tissue of the head of another specimen, although they were not found in its brain.

The next examination was conducted in August and spores were found in the bone-cartilage tissue of the head of all 30 specimens that were examined. Infestation was severe in 18 specimens, moderate in four, and mild in intensity in eight specimen. An examination of the brains of these trout revealed spores in 25 cases in mild intensity, while the findings in the other organs were negative.

In October we found spores of Myxosoma cerebralis in the cartilage-bone tissue in all 30 specimens examined. Among these trout, the infestation was severe in 18, moderate in one, and mild in intensity in 11 cases. Moreover, in one trout spores were found in all of the organs that were examined; in the spleen and liver of four trout, in the contents of the intestine in two, and in the kidneys of eight.

The December study revealed infestation of severe intensity in the cartilage-bone tissue of the head in all of the specimens examined. Severe spore invasion was found in the brains of seven trout; moderate in 15; and mild in eight specimens. Spores were found in the spleen of five specimens, in the livers of two, and in the kidneys of three trout.

Graph 1 shows the percentage representation of finds of spores of M. cerebralis in the organs that were examined.

Discussion

The percentage total of finds of spores of M. cerebralis in individual organs of the total number of specimens that were examined indicates that the bone-cartilage tissue of the head and the brain are fully reliable diagnostic tissue, considering the spore findings in

them of 86.7 and 71.2 percent, respectively. As opposed to these tissues, the kidneys with 14.4 percent, the spleen and liver with 10 percent, and the contents of the intestine with 2.2 percent positive spore findings are tissues and substrata that cannot be used alone in diagnosis, but which could also be of some use as supplementary elements in confirming the presence, extent, and degree of infestation with spores of M. cerebralis.

The results that are presented in the graph on the detection of spores of M. cerebralis in various organs and which are based on four randomly selected samples of 30 trout each, taken in the interval from /62 July to December, show that besides the cartilage-bone tissue (86.7 percent), spores are most frequently found in the brain (71.2 percent). The detection of spores in 2.2 percent of the cases in the contents of the intestine only partially supports the earlier thesis that the path of elimination of spores from the organism is by way of the intestine [Uspenskaya et al.]. In the epizootiology of myxosomatosis, such a low level of infestation of the intestine with spores combined with a very low intensity (one or two spores) is in practical terms more of theoretical value. The results that have been obtained are similar to the findings of other authors, except for the brain, for which we were unable to find data in the available literature. The reason for this is probably the fact that most authors utilize the bones of the head together with the brain when they are examining juveniles for myxosomatosis. According to our observations, diseased fish very frequently exhibit exophthalmos and these same fish are significantly less vital; consequently, they end up lacking one or both eyes. Since the eye sockets communicate directly with the cavity of the crenium, it is the soft parts that are the first to decay when

a dead fish rots. This means that the spores reach the water together with the brain mass by way of the eye socket. These suppositions are supported by the fact that the brain mass is the most severely infested of all the tissues except for the cartilage-bone tissue in terms of intensity of infestation.

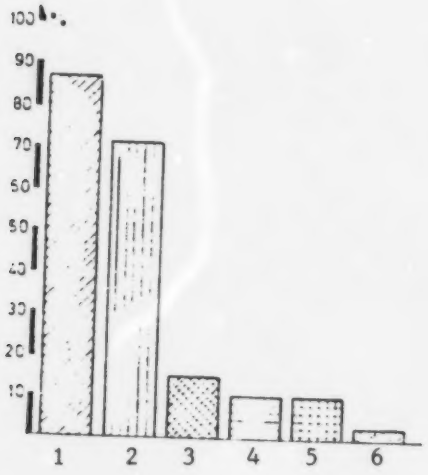
Conclusion

During an investigation of the frequency of findings of Myxosoma cerebralis spores in different organs in 120 specimens of juvenile California trout with clinical symptoms of myxosomatosis, positive findings were obtained by the native preparation method: in 86.7 percent of the specimens when examining the cartilage-bone tissue; 71.2 percent in the case of the brain; 14.4 percent, the kidneys; 10 percent, the spleen and the liver; and 2.2 percent, in the contents of the intestine.

As the juveniles grow older both the extensiveness and the intensity of infestation in the cartilage-bone tissue increase, as does the percentage of spore presence in the other organs.

The detection of spores in the brain to the extent of 71.2 percent is a new contribution to greater knowledge of the epizootioloty and the source of infestation with M. cerebralis spores.

GRAPH 1



Spores of M. cerebralis found in the different organs of California trout:

1) bones of the head
2) brain
3) kidneys
4) liver
5) spleen
6) contents of the intestine

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OCCURRENCE OF MYXOSOMA CEREBRALIS SPORAE IN THE ORGANS OF CALIFORNIAN TROUT

Summary. — *Aiming at acquiring a better knowledge of means and ways of infestation, i.e. acquiring and improved knowledge of epizootiology of Muxosomiasis in trout, an investigation was conducted of occurrence of Myxosoma cerebralis in different organs in 120 young Salmo g. irideus Gibb. which manifested clinical forms of the disease. In 4 random samples, each containing 30 fish, over the period from july through december, by means of native preparation, a positive finding was obtained from the head cartilage-bone tissue in 86,7% of the fish, from brain in 71,2%, from kidneys in 14,4%, liver and spleen in 10%, and intestine contents in 2,2% of the examined young.*

As young fish grow older the extensivness and intensity of infestations in cartilage-bone tissue of the head increase, as does the per cent of cases of spora presence in other organs.

Detecion of sporae M. cerebralis in the brain, with the intensity of 71,2%, is seen as a new contribution to improving knowledge of epizootiology and source of infestation with sporae M. cerebralis.